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Effects of Acidic Rain and Ozone on Nitrogen Fixation and Photosynthesis in the Lichen 'Lobaria pulmonaria' (L.) Hoffm

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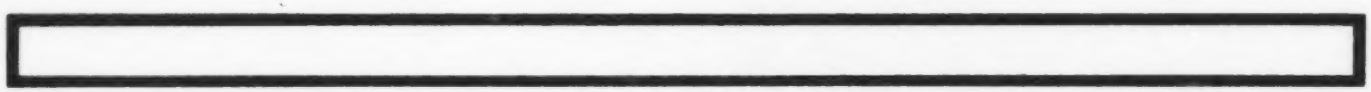

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16. ABSTRACT
The lichen Lobaria pulmonaria was subjected to ozone fumigations at 118, 235 and 353 ug/m^3 and simulated acidic rain at pH levels of 2.6, 4.2 and 5.6 for 5 days (M,W,F,M,W) during a 10-day period. Acidic rain at pH 2.6 caused significant reduction in nitrogen fixation and gross photosynthesis of 100 and 90%, respectively, and thallus bleaching was apparent. There were no significant differences between the pH 5.6 and 4.2 treatments in either gross photosynthesis or nitrogen fixation, and the color of the lichen thalli was unchanged. The effect of O_3 on nitrogen fixation and photosynthesis over the range of concentrations used was not significant, but there was a trend toward reduced nitrogen fixation with increasing O_3 concentration. There were no significant O_3-acidic rain interactions. The threshold for response to rain acidity for L. pulmonaria lies between pH 2.6 and 4.2, and the acidity of wet deposition in parts of the United States may fall in this range.

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EFFECTS OF ACIDIC RAIN AND OZONE ON NITROGEN FIXATION AND PHOTOSYNTHESIS IN THE LICHEN *LOBARIA PULMONARIA* (L.) HOFFM.

LORENE L. SIGAL and J. WILLIAM JOHNSTON, Jr.

Environmental Sciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, U.S.A.

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SIGAL L. L. and JOHNSTON J. W., Jr. *Effects of acidic rain and ozone on nitrogen fixation and photosynthesis in the lichen* Lobaria pulmonaria *(L.) Hoffm.* ENVIRONMENTAL AND EXPERIMENTAL BOTANY **26,** 59–64, 1986.—The lichen *Lobaria pulmonaria* was subjected to ozone fumigations at 118, 235 and 353 $\mu g/m^3$ and simulated acidic rain at pH levels of 2.6, 4.2 and 5.6 for 5 days (M, W, F, M, W) during a 10-day period. Acidic rain at pH 2.6 caused significant reductions in nitrogen fixation and gross photosynthesis of 100 and 90%, respectively, and thallus bleaching was apparent. There were no significant differences between the pH 5.6 and 4.2 treatments in either gross photosynthesis or nitrogen fixation, and the color of the lichen thalli was unchanged. The effect of O_3 on nitrogen fixation and photosynthesis over the range of concentrations used was not significant, but there was a trend toward reduced nitrogen fixation with increasing O_3 concentration. There were no significant O_3–acidic rain interactions. The threshold for response to rain acidity for *L. pulmonaria* lies between pH 2.6 and 4.2, and the acidity of wet deposition in parts of the United States may fall in this range.

INTRODUCTION

BECAUSE lichens obtain their nutrients from rain and air, they are especially vulnerable to changes in air quality and thus may be useful as early indicators of environmental change. Their response to sulfur dioxide (SO_2) is well documented,[17] but reports on the effects of ozone (O_3) or acidic rain on lichens are few. Field studies show that many species once found in the highly oxidant-impacted areas of the San Bernardino and Santa Monica mountains in California are now absent.[20,25] Results of laboratory studies with O_3 range from no effects on photosynthesis in *Cladonia rangiformes* Hoff. at O_3 concentrations as high as 11,755 $\mu g/m^3$ for 2.5 hr[4] to significant reductions in photosynthesis in *Parmelia sulcata* Tayl. and *Hypogymnia entermorpha* (Ach.) Nyl. at O_3 concentrations of 980 and 1567 $\mu g/m^3$, respectively, after 3 hr[15] and *Pseudoparmelia caperata* (L.) Hale at 200 $\mu g\ O_3/m^3$ after 12 hr.[21]

Effects of low pH on lichens have been determined primarily by the use of buffer solutions in studies to determine the effects of SO_2.[1,8,17] More recently, LECHOWICZ[12] found that photosynthesis in *Cladina stellaris* (Opiz.) Brodo was reduced by treatment with simulated rain at pH 4 in the laboratory. Natural mats of the same species were treated with simulated rain solutions at pH values of 2.5, 3.0, 3.5, 4.0, 4.5, 5.0 and 5.6. The solutions were adjusted to 2:1 and 6:1 ratios of sulfate to nitrate with sulfuric and nitric acids. After 2 years, there were no significant effects on the growth rate of the lichen, but thallus discoloration was apparent below pH 3.5. After 3 years, lichen growth was significantly depressed in specimens treated with the sulfate-rich solutions (6:1) below pH 4.5.[13] BAILEY and LARSON,[2] however, found that pH and water quality had little

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effect on the rates of carbon dioxide (CO_2) exchange in *Umbilicaria mammulata* (Ach.) Tuck. Little is known about the effects of O_3 or acidic rain on lichens capable of fixing nitrogen (about 55 species); yet these lichens may contribute significantly to the nitrogen budget of forested ecosystems.[3,16,18] Denison *et al.*[6] reported a trend toward decreased nitrogen fixation in the lichens *Lobaria pulmonaria* and *Lobaria oregana* (Mull. Arg.) Hale as a function of decreasing pH of the water in which the lichens were soaked. Several experiments dealing with the effects of SO_2 on nitrogen fixation and photosynthesis in lichens show that nitrogen fixation may be a more sensitive indicator of stress than photosynthesis.[7,11] In these experiments, photosynthesis was reduced by 0–50% and nitrogen fixation by 80–97%.

There are currently no reports about the interactive effects of O_3 and acidic rain on lichens, nor are there sufficient data to determine if these pollutants affect nitrogen-fixing species of lichens. In the present study, *L. pulmonaria*, a nitrogen-fixing lichen found in mesic forests of the eastern and western United States, was exposed to O_3 and/or simulated acidic rain (a) to determine threshold concentrations for effects of O_3 and simulated acidic rain on photosynthesis and nitrogen fixation, and (b) to test for interactions between O_3 and simulated acidic rain.

MATERIALS AND METHODS

Experimental design

The experiment to determine the thresholds and interactive effects of O_3 and simulated acidic rain on *L. pulmonaria* used a 4 × 3 factorial design and was repeated 3 times. The data were analyzed with the GLM procedure of SAS[22] with repeat treated as a blocking variable. The basic experimental unit for analysis was the mean response within a repeat. Significant differences between means were determined by LSD tests when warranted by significant results from *F*-tests.

Collection and storage

Lobaria pulmonaria thalli were collected from oak trees (*Quercus* spp.) at the Oak Ridge National Environmental Research Park, Roane County, Tennessee, on 19 October and 2 and 16 November 1983. Each collection was stored in a growth chamber with an 11 hr photoperiod [115 μmol/m^2/sec photosynthetic photon flux density (PPFD)]. Day and night temperatures were maintained at 20 and 10 ± 1.0°C, respectively. The lichens were wetted twice with distilled water during a 3-day acclimatization period prior to treatment.

Treatments

Thalli were exposed to O_3 and simulated rain on 5 days (M, W, F, M, W) during a 10-day period. The O_3 exposures were conducted in a continuously stirred tank reactor system described by Taylor *et al.*[27] Ozone was produced by passing O_2 over an ultraviolet light source (Monitor Labs ozone generator) and was diluted with charcoal-filtered air to maintain concentrations of 0, 118, 235, or 353 μg O_3/m^3 ± 10% (1 ppm = 1 967 μg/m^3). Chamber concentrations were monitored with a chemiluminescence ozone monitor (Monitor Labs Model 8410). Light was supplied with 1000 W multivapor lamps (230 μmol/m^2/sec at the thalli surface). Temperature and relative humidity averaged 26°C and 34 ± 10%, respectively. Prior to exposure and hourly during the 4 hr O_3 exposure, the thalli were misted with distilled water. Two hours after termination of the O_3 fumigation, the lichens were exposed to simulated rain (1.1 cm, 40 ± 5 min) with pH adjusted with sulfuric acid (H_2SO_4) and nitric acid (HNO_3) in a 1.5:1 molar ratio to 5.6, 4.2, or 2.6 on a greenhouse rain simulator.[24] Rain simultants mimicked the ionic concentrations normally found in rain in the continental eastern United States, excluding heavy metals.[9] Between exposure days (~ 48 hr), lichens were stored as described above and allowed to dry.

Measurements of nitrogen fixation and gross photosynthesis

On the 2 days following the final exposures, thallus tips (~36 mm^2) were used for $^{14}CO_2$-uptake assay, and thallus lobes with similar tip-to-center ratios (85–475 mg oven-dried sampleweight) were used for nitrogen fixation assays.

Nitrogen fixation was assayed with the acetylene reduction technique of STEWART *et al.*[26] The lichen thalli were soaked for 1 hr in distilled water, blotted dry, and put into 50-ml test tubes fitted with serum stoppers. Five milliliters of air was withdrawn with a syringe and replaced with 5 ml of acetylene (C_2H_2). Prior to injection, the acetylene was bubbled through concentrated H_2SO_4 and distilled water to remove ethylene impurities. The test tubes were incubated for 4 hr in a growth chamber (20°C ± 10%, 315 μmol/m^2 sec PPFD) and shaken twice during the incubation period to enhance mixing. Preliminary assays with untreated lichens showed that C_2H_2 reduction was linear during the 4-hr incubation.

After incubation, 1-ml samples were withdrawn in disposable syringes and analyzed for ethylene (C_2H_4) with a gas chromatograph (Perkins Elmer Model 39), equipped with a flame ionization detector and a 1.8 m × 3.2 mm (6 ft × 1/8 in.) column of 80/100 Poropak R. The column was operated at 38°C; helium (80 ml/min) was the carrier gas. Peak heights and amounts of C_2H_2 and C_2H_4 were determined with a Hewlett Packard 3380A reporting integrator. Acetylene was used as an internal standard. Results were expressed in nanomoles of C_2H_4 per gram of lichen thallus (dry weight) per hour. There were three replicate samples for each treatment.

Gross photosynthesis was determined with a porometer, similar to that described by SHIMSHI,[23] in a growth chamber at 20°C ± 10% and 315 μmol/m^2/sec PPFD. Thallus tips were exposed to a single 30-sec pulse of $^{14}CO_2$ in a cuvette. Immediately following exposure, the tips were placed in scintillation vials on dry ice, oven dried for 24 hr at 80°C, weighed, and combusted in a Packard Tri-carb (Model 306) sample oxidizer. The amount of ^{14}C in the sample was determined by standard liquid scintillation procedures. Gross photosynthesis was reported as disintegrations per min (dpm) per mg (dry wt) of thallus. There were six replicate samples for each treatment.

RESULTS

The effect of repeat was statistically significant for gross photosynthesis, but not for nitrogen fixation (Table 1). Gross photosynthesis was lower in repeat 1 than in repeats 2 and 3. The difference may have been due to variation in environmental factors to which the lichens were exposed either prior to collection or in the greenhouse environment during acid rain exposures. The lack of interaction between the blocking factor (repeat) and main effect factors was assumed in the analysis of the data.

Table 1. Response of Lobaria pulmonaria *to* O_3 *and acidic rain: analysis of variance*

		Photosynthesis		N-fixation	
Source of variation	d.f.	Mean square ($\times 10^3$)	$P(>F)$	Mean square ($\times 10^6$)	$P(>F)$
Repeat	2	305	0.01	10.3	0.12
Ozone	3	63	0.38	5.3	0.33
Rain	2	14,735	<0.01	12.3	<0.01
Ozone–rain	6	46	0.59	3.6	0.56
Error	22	59		4.4	

Simulated acidic rain at pH 2.6 caused significant (Table 1) reduction in gross photosynthesis (Fig. 1) and nitrogen fixation (Fig. 2), with reductions of 90 and 100%, respectively. In addition, the lichen thalli exposed to pH 2.6 rain developed a yellowish cast. There were no signi-

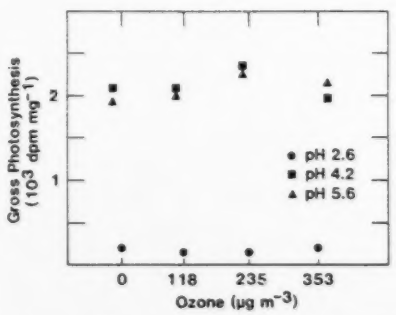

FIG. 1. The effects of O_3 and acid rain on gross photosynthesis in the lichen *Lobaria pulmonaria*. S.E.M. = 1.4×10^2.

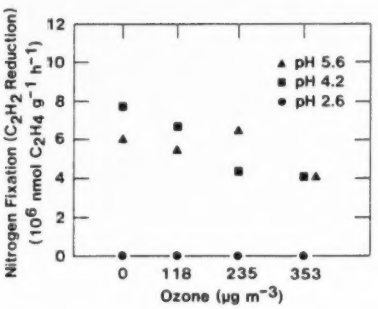

FIG. 2. The effects of O_3 and acid rain on nitrogen fixation (i.e. acetylene reduction) in the lichen *Lobaria pulmonaria*. S.E.M. = 1.2×10^3.

ficant differences between the pH 5.6 and 4.2 treatments in either gross photosynthesis or nitrogen fixation, and the color of the lichen thalli was unchanged.

The effect of O_3 on nitrogen fixation and photosynthesis over the range of concentrations that were used was not significant (Table 1). Examination of the means indicates a trend toward reduced nitrogen fixation with increasing O_3 concentration (Fig. 2). There were no significant O_3–acidic rain interactions (Table 1) associated with gross photosynthesis or nitrogen fixation.

DISCUSSION

The concentrations of O_3 and the pH levels in the simulated acidic rain used in this study are representative of atmospheric conditions across the United States. For instance, 235 $\mu g/m^3$ of O_3 is the National Ambient Air Quality Standard, but it is exceeded in many regions of the country, particularly in southern California.[5,28] The hydrogen ion (H^+) content of the simulated rain treatment at pH 2.6 is similar to the more extreme H^+ concentrations found in fog and cloud water and in occasional low-pH rainfall. For example, the median acidity for fog samples taken during more than 20 days in southern California corresponded to a pH of <3;[30] LOVETT *et al.*[14] reported a mean H^+ concentration in New Hampshire cloud water, which corresponded to a pH of 3.54. The pH 4.2 and 5.6 rain treatments are typical of median pH levels for much of the northeastern, midwestern, and western parts of the United States.[29]

The threshold for significant reduction of photosynthesis and nitrogen fixation in *L. pulmonaria* is between pH 4.2 and 2.6. As shown above, the acidity of wet deposition in some areas of the United States may fall in this range.

Our laboratory results are similar to those of DENISON *et al.*[6] who found that treatment of *L. pulmonaria* with water acidified with sulfuric acid to pH 2 almost completely eliminated nitrogen fixation, whereas pH levels of 4–8 had no significant effects. Evidence for effects of acidic rain on nitrogen-fixing lichens in the field is circumstantial and confounded by the presence of multiple pollutants. In the United Kingdom, where the annual average pH of precipitation is 4.2, ROSE[19] reported that lichens in the *Lobarion* community, which is composed of many nitrogen-fixing species,[10] are now restricted to ash (*Fraxinus* spp.) and elm (*Ulmus* spp.) trees. These trees have a more highly buffered bark than the oaks, which were the major habitat for the community in the past. Our results show that levels of acidity in rain and fog may be sufficient to negatively affect physiological processes of *L. pulmonaria*. However, recovery processes must be studied before physiological responses in the laboratory can begin to be linked to changes in lichen distribution in the field.

The lack of an O_3 effect suggests that photosynthesis and nitrogen fixation in *L. pulmonaria* may be tolerant of low O_3 concentrations likely to be encountered in the eastern United States, but the concentrations used in this experiment are not sufficiently high to evaluate the potential effects of the elevated levels of O_3 in the west. The tendency toward reduced nitrogen fixation in response to 353 $\mu g/m^3$ of O_3, for which the statistical significance was masked by high variability, requires further study. Since there was no O_3 effect, conclusions concerning the interaction between O_3 and acidic rain are limited to the effects of subthreshold O_3 doses on the physiological response to rain acidity.

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